PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#24/W.M. 5/8/03

In re application of

KAYYEM

Serial No.: 09/295,691

Filed: 4/21/1999

For: *THE USE OF MICROFLUIDIC SYSTEMS IN THE ELECTROCHEMICAL DETECTION OF TARGET ANALYTES*

Group No. 1743

Examiner: STARSIAK, JOHN S

CERTIFICATE OF MAILING
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Sir:

I, **Jon F. Kayyem**, do hereby declare as follows:

1. I am an inventor on the above identified patent application and am familiar with its contents.

2. I have reviewed the pending claims in this application.

3. I am familiar with the Office Action mailed on January 13, 2003, where claim 36 was rejected under 35 U.SC. 103(a) as being unpatentable over Segal (U.S. Patent 6,300,141) filed on March 2, 2000, claiming priority to Provisional Application No. 60/122,546, filed on March 2, 1999.

4. That, prior to March 2, 1999, as evidenced by notes from a meeting, a copy of which is attached as Exhibit 1, the basic concept of a microfluidic device for detection of target analytes was conceived. The notes attached as Exhibit 1 were taken at a meeting prior to March 2, 1999. The meeting included myself, Dr. Gary Blackburn, the Director of Scientific Affairs (who took the notes), and patent counsel, Robin M. Silva and Richard F. Trecartin. These notes indicate that the combination of microfluidics with CMS technology was discussed; see arrows on page 1 and 2. In particular, the discussion of microfluidics included separation and sample preparation; see arrow on page 1.

5. That, as evidenced by Exhibits 3-5, "CMS technology" prior to March 2, 1999 included at least a detection well having a detection electrode comprising a self-assembled monolayer and a capture binding ligand. Exhibit 2 is an article, published prior to March 2, 1999 by Daniel H. Farkas "Clinical Micro Sensors developing point-of-care molecular diagnostics". The article attached as Exhibit 2 demonstrates that CMS microchips contain numerous electronically acrive "pads," each embedded with specific DNA capture probes (see col. 1, paragraph 2). The article further discloses that the microelectrode surface is electrically insulated with a monolayer coating of alkane thiols (see col. 2, paragraph 2). Exhibit 3 is an

article, published prior to March 2, 1999, that also discloses that CMS microchips contain electronically active pads containing specific DNA probes and insulated for protection with a thin coating of organic molecules (see col. 2, paragraph 2). Exhibit 4 is a Press Release, dated prior to March 2, 1999, disclosing that CMS chips use electronic detection of DNA at electrode surfaces modified with self-assembled arrays (see paragraph 4). Exhibit 5 is a copy of E. K. Wilson, "Instant DNA Detection" *Chemical and Engineering News*, vol. 76, No. 21, 1998. The last figure ("Molecular 'Wire' connects DNA complex to electrode in the CMS system") depicts an electrode as used in a CMS system, comprsing a self-assembled monolayer and a capture probe.

6. "CMS technology" prior to March 2, 1999 further included an inlet port and a channel between the inlet port and the detection well, as evidenced by FIG. 1 in attached Exhibit 2. The Figure shows a detection well, an inlet port, and a channel between the detection well and inlet port. An enlarged copy of the figure, with those portions labeled is attached as Exhibit 6.

7. That, prior to March 2, 1999, as evidenced by a letter from Robin M. Silva, a copy of which is attached as Exhibit 7, I received a draft of the present patent application for review and comment.

8. That, between March 2, 1999 and the April 21, 1999 filing date of the present application, I worked diligently with patent attorney Robin M. Silva to finalize and file the present application.

9. That, in conclusion, there was a conception of the invention coupled with due diligence prior to and extending through March 2, 1999, the effective date of U.S. Patent No. 6,300,141, leading to a constructive reduction to practice in the United States by filing the parent application Serial No. 09/265,691 on April 21, 1999.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made herein with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 in the United States Code, and that any such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Date: April 15, 2003

Signed: _____
Jon F. Kayyem

SF_1108761v1

Exhibit 1

Robin Neil, Pat

Strategy Mtg

Signal Processing
Sample Prep
MEMS
ECL
Hybridization
Accelerometer

Robin

Gold Str Af
SAM's Str Af
hardware
software - signal processing
CJ new chemistries
microfluidics
a) separation
b) sample prep
sample prep
Cindy's new-1 invention (collard)
Robin will project costs forward 12-24 months

Gold Str

trade secret vs patent?
should be broader than pcb

SAM Shoff

Aqueous
would be very important for manufacturing

Hardware -

Sensitivity (MOx - Jerry Discroll
Probant unique

Signal Processing

Harmonics
Phase + harmonics
Changed wave form
Creager professional ~ April 98
Roger Doering is working on application.

CJ's new chemistry

branched DNA
ferrocene polymers (filed already)

novel nucleotides
(eg Enzo alternatives)

Faiz - important since we may be
a "chip" company (eg w/ Motorola)

Microfluidics

Combination w/ CMS
Faiz - "on future"
"the detector for microfluidics"
"cart & parts"

Sample Prep - eg Cindy prep protocol
for DARPA

Cindy's Collaborative invention

Exhibit 2

Clinical Micro Sensors developing point-of-care molecular diagnostics

DANIEL H. FARKAS

Clinical Micro Sensors Inc. (CMS) was organized in 1995 to capitalize on a bioelectronic DNA detection technology developed at the California Institute of Technology by the company's founder, Jon Faiz Kayyem, with Thomas Meade. The essence of the technology rests on specific detection of the electrical current generated by the reversible and continuous oxidation and reduction of labeled nucleic acid targets. Refinements of this system will have wide-ranging applications in clinical diagnostics, blood-safety monitoring, food safety, biomedical research, pharmacogenomics, and the detection of biowarfare agents.

The CMS technology exploits the electronic properties of nucleic acids while relying on the central principle of clinical molecular diagnostics, namely, hybridization. In the CMS technology, the solid support used as the original tool of molecular diagnostics—the nitrocellulose or nylon membrane of the Southern blot—is replaced by a DNA microchip. CMS microchips contain numerous electronically active "pads," each embedded with specific DNA capture probes. The number of pads and specificity of probes on a chip can be varied depending on the applications of interest. The chips also include redundancy for positive controls and nonsense sequences as negative controls.

To serve as an electron donor, a ferrocene organometallic complex is covalently attached to the single-stranded DNA capture probes, or to a signal probe that is complementary to a different region of the target of interest. The signal probes serve to label the target upon hybridization and are called AMBER probes (AMBER is an acronym for amperometric bioelectronic reporter). The capture probes are attached to a gold microelectrode through phenylacetylene "molecular wires" that maintain the desired electrical contact between the probes and the surface of the gold electrode. Except for these protruding molecular wires, however, the microelectrode surface is electrically insulated with a monolayer coating of alkane thiols. This coating prevents unwanted redox species in the sample chamber of the chip from interfering with the measurements of the test system. Generation of a signal, therefore, depends on specific probe-target interaction—that is, hybridization—which is transmitted through the molecular wire.

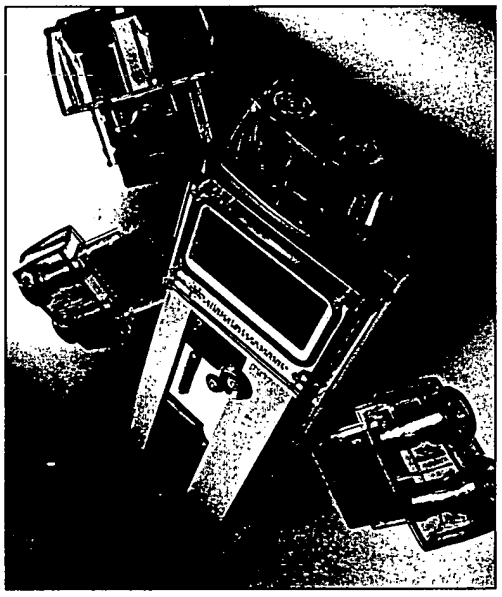

Figure 1. Working prototype of the CMS handheld reader. The reader applies microvoltage to a DNA microchip to initiate sensing (one such chip is shown inserted in the top of the reader and three others appear in the photo). Test results are generated in minutes and are displayed on the reader's built-in screen. The reader also includes an infrared port for data transmission, which appears as a small, dark oval to the right of the inserted chip.

When a slight voltage (in the millivolt range) is applied to the sample-containing microchip following hybridization, the ferrocene labels release electrons that rapidly travel through the double-stranded nucleic acid hybrid and molecular wires, yielding an electronic signal that can be detected through the microelectrode. CMS's proprietary handheld reader supplies the voltage that initiates these events, and has onboard software that interprets the electrical signal to both identify and quantitate, if appropriate, the target nucleic acid (see Figure 1).

Molecular diagnostics is a burgeoning field that will ultimately bring dramatic changes in the ways that laboratory medicine and clinical diagnostics are practiced. In 1999, however, molecular diagnostics represent only a tiny fraction of the total volume of diagnostic tests performed in hospitals, and an even smaller percentage of those performed in physician office laboratories. There are several reasons for such low volumes of molecular testing. The amplification procedures that define the gold standard of molecular diagnostics (e.g., polymerase chain reaction, ligase chain reaction, and so on) are complex, time-consuming, and expensive, and often require not only dedicated space but also the most highly trained, specialized medical technologists. Furthermore, enzymatically based amplification methods can be inhibited by certain components of the specimen under analysis. Relatively complicated and expensive specimen preparation technologies must often be used to prepare patient specimens for molecular diagnostic tests.

PHOTO BY HENRY BLACKHAM

Extensive experimentation at CMS has demonstrated that the bioelectronic detection of DNA described above is not inhibited by the presence of serum, whole blood, saliva, or urine, and can even be performed on such samples as soil extracts and hamburger. The CMS technology, therefore, can be adapted to applications in molecular diagnostics and other markets without the need for extensive specimen preparation. Sample preparation—consisting of simple lysis of the cells, bacteria, or viruses of interest followed by denaturation and fragmentation—will not need to be augmented by significant purification. Ultimately, the company intends to integrate lysis of the specimen and presentation of the nucleic acid into the system. A complete test will then be performed by introduction of the specimen, a few moments of incubation for lysis and nucleic acid denaturation and fragmentation, several minutes of incubation for hybridization to occur, signal generation (initiated by pushing one button to generate the necessary voltage), and reading the result displayed on the device.

The CMS DNA microchips are inexpensive to manufacture, resulting in cost savings that will help to make molecular diagnostics more widely available. Progress on the sensitivity levels of the system has been dramatic. For many current applications, detection levels of 1000 to 100,000 targets is satisfactory. As CMS moves toward even more-sensitive detection levels, the requirement for enzymatic nucleic acid amplification may be eliminated or reduced. Coupling CMS's technology to microfluidics may also be useful in improving sensitivity by concentrating specimens.

CMS's chips contain electronic pads embedded with DNA capture probes.

The potential speed, sensitivity, ease of use, and low cost of CMS's bioelectronic DNA detection system are all factors that will contribute to its broad adoption as a new platform for nucleic acid detection. In medical applications, the system could enable physicians to administer antibiotics or antivirals rationally and specifically with point-of-care availability of results. CMS chips could comprise panels that cover the entire differential diagnosis associated with given symptom sets, such as respiratory infection panels, meningitis panels, sexually transmitted disease panels, and so on. The CMS platform could also be adapted to instruments designed for the central testing laboratory, enabling laboratorians to assay many patient specimens per batch.

Daniel H. Farkas, PhD, is director of clinical diagnostics at Clinical Micro Sensors Inc. (Pasadena, CA).

making substantial progress. According to Mimms, current technologies enable manufacturers to fabricate single-chip arrays containing roughly 20,000 wells, with each well holding about 100 nl. One approach to attaining high densities is being studied by researchers at the National Institute of Standards and Technology, who have developed a chip made up of a single-molecule layer of DNA bound to a thin film of gold. The surface-tethered DNA then binds to a target strand and piezoelectric differences signal the level of hybridization. Tiny electrical charges, infrared spectroscopy, and scanning tunneling microscopy help to organize the ultrathin layers. Other researchers have displayed remarkable results with elastomer films. In fact, the progress in this area is so encouraging that Harvard University scientist George Whitesides has declared that "the days of silicon-based chips are numbered."

What is the theoretical limit of such arrays? That is a question routinely asked in this new field, as the scale of operations becomes ever smaller. One guess puts the theoretical boundary at 1 million elements on a 1-cm^2 chip. Norman Nelson, senior research scientist at Gen-Probe, points out that "problems with hybridization worsen at higher densities. And there also is a point where the optics of detection reach their limits of resolution."

Integrated Chips. The final area of development is in the integration of sample preparation and analysis—the so-called lab-on-a-chip technology. "Today, all companies in this field recognize the importance of integration and are working on it," says Peter Wilding, director of clinical chemistry at the University of Pennsylvania. "To achieve this, these chips need to have a microfluidics platform."

"In order for chips to reach their full potential, they should evolve to include sample preparation, analysis, and signal acquisition," agrees Larry Kricka, also a professor in the pathology department at the University of Pennsylvania. "This form of a lab-on-a-chip will have many potential applications in both clinical and nonclinical areas."

Manufacturers are pursuing a variety of strategies to improve the microfluidics capabilities of chips. "Conventional capillary electrophoresis (CE) has been used to perform a diversity of analyses involving proteins, nucleic acid fragments, drugs, and so on. Such flexibility is a compelling reason for considering this technique for on-chip detection," explains Kricka. "CE and laser-induced fluorescence have been particularly popular for separation-detection combination. But other techniques have also been explored, including on-chip chemiluminescence, and electrochemiluminescence assays."

Not everyone agrees that lab-on-a-chip technologies are the coming wave, however. "I don't think the field will need to evolve to lab-on-a-chip," says Gen-Probe's Nelson. "Clinical laboratories will be willing to perform the preparation and analytical steps off-line, or by using a more extensive instrument." What then becomes critical, he and others add, is that DNA-chip technologies be compatible with existing equipment in such laboratory settings.

PHOTO COURTESY INCYTE PHARMACEUTICALS

CLINICAL MICRO SENSORS INC.

Exhibit 3

Inexpensive, convenient DNA chips for infectious and genetic diseases

126 West Del Mar Boulevard
Pasadena, CA 91105
Phone: (626) 584-5900
Fax: (626) 584-0909

- Contact: Jon F. Kayyem, PhD, President & CEO
- Industry Segment: Drug discovery and molecular diagnostics
- Business: Low-cost, simple-to-use DNA microchips
- Founded: 1995
- Founder: Jon Kayyem, PhD
- Employees: 40
- Financing to date: $14 million
- Scientific Advisory Board: Thomas Meade, PhD (California Institute of Technology), Scott Fraser (Beckman Institute; California Institute of Technology), Harry Gray (Beckman Institute, California Institute of Technology)

As a graduate student at the **California Institute of Technology** (Caltech) in the early 1990s, Jon Kayyem heard LeRoy Hood, molecular biologist, lecture about the significance of the Human Genome Project as he urged scientists to participate in it. Kayyem wasn't interested in studying genes, but he was looking to develop practical applications for the information gleaned from this basic work.

But chip technology, so critical to biomedical research applications such as gene discovery and mapping, seemed too complex and expensive for clinical use. Kayyem also looked at various detection technologies, but found them all problematic. The common technique of labeling probes with a fluorescent signal and other light-based methods had drawbacks, such as complexity and difficulty in reducing background noise.

An associate of Kayyem's and member of the research faculty at Caltech, Thomas Meade, PhD, was studying DNA's abilities to conduct electricity, and the two scientists together developed a method of initiating and measuring electrical current through DNA. Recognizing the significance of this discovery, Kayyem negotiated with Caltech for a license to the technology in exchange for a small stake in the newly founded **Clinical Micro Sensors Inc.** [W#952657]. Kayyem reasoned that if electrical signals could be used in assays to detect DNA, they would be cheaper than light-based or other methods. The goal of the new company was to develop a DNA microchip with electronic detection capabilities, which would be versatile, easy-to-use, inexpensive and would appeal to a variety of customers.

CMS microchips contain numerous—20 or more—electronically active pads, i.e. electrodes. The pads contain specific DNA probes and are insulated for protection with a thin coating of organic molecules. The coating contains a series of molecular wires which attach the probe sequences to the pads. When a sample is introduced into the device, it is subjected to a simple chemical treatment that causes human cells, bacteria and viruses to burst open or lyse. In this way, the target DNA is made available for hybridization. If the probe DNA encounters complementary target DNA from the sample, hybridization occurs. When a slight voltage is applied to the sample following hybridization, the metal labels on the probes release electrons that move rapidly through the molecular wires, completing a circuit. This resulting signal is read by a reader and can be used to identify and quantify any genetic sequence, including those of viral and bacterial pathogens.

The bio-electronic detection method will allow CMS chips to achieve clinically relevant levels of detection for many applications, eliminating the need for labor-intensive, expensive target amplification, Kayyem says. Target amplification technologies, of which the most popular is PCR (polymerase chain reaction), are required for most present DNA-based diagnostics and DNA microchip products. But, because the metallic labels can be packed together on a probe, they can detect the presence of minute amounts of DNA in a sample (a form of signal amplification) and therefore often don't require target amplification. If fluorescent labels are crowded densely together, on the other hand, they interfere with each other, clinching the signal. Thus, target amplification is often required to pick up signals from small amounts of DNA in a sample, says Kayyem.

The chips incorporate other labor-saving technologies: the coating on the electrodes filters out non-specific signals, allowing only specific molecules that the user wants to detect to participate in the reaction. This eliminates the need for a wash step. Moreover, current DNA chips require large lasers and scanners to read the signals emanating from the reaction. These machines are too costly for use in clinical or industrial settings. CMS chips can be read by an inexpensive reader, which has been adapted to a battery-powered, hand-held point-of-care design. CMS's software also filters out unwanted noise to detect the presence of target DNA with minimal sample preparation.

CMS's approach to marketing its biochip and reader is similar to Intel's business model prior to the mass commercialization of personal computers. Its aim is to make the biochip a platform for manufacturers, which will integrate them into finished products for sale to end-users. CMS's likely customers are diagnostics manufacturers, which will use the chips for infectious disease testing, and pharmaceutical companies for drug discovery and genomics research, as well as assorted industrial testing companies (food safety, agriculture, environmental monitoring), and animal health and forensics facilities. The first products are expected to be ready for commercialization in two years for non-clinical applications and in 2002 for

CONTINUED ON PAGE 28

Clinical Micro Sensors, *continued*

clinical diagnostics.

Pharmaceutical companies are already working with biochips, but CMS chips are much less expensive, says Kayyem. Clinical laboratories would eventually be likely users of the diagnostics chips for testing, but they are traditionally conservative about changing platforms and their conversion to new technologies is often slow. The technology is versatile and cheap enough to be practical for physicians' offices once CMS gets the chip costs down from the current $7 per unit to $1-2 per unit, something Kayyem thinks is feasible in the near term as the company converts from a manual to an automated manufacturing process.

In addition to raising an initial $6.9 million from private investors [W#953395], CMS has an $825,000 grant from the Department of Energy's Small Business Innovation Research program, a $1.8 million grant from the Department of Defense, and a $2 million grant from the Advanced Technology Program of the Department of Commerce. Moreover, it just closed the first part of a $15 million private placement, raising $7 million [W#983334].

Kayyem, the president and CEO, is a co-inventor of the Caltech patent and several subsequently issued patents. He has a PhD from Caltech, where he was a member of the faculty, and spent three years researching electron transfer through DNA. Gary Blackburn, PhD, is VP, scientific affairs. Previously, he was director of technology management at Boehringer Mannheim Corp. (now merged with **Roche**), where he was responsible for the licensing of new technologies. Prior to that, he was director of R&D at **Igen International Inc.** Daniel Farkas, PhD, is director of clinical diagnostics and was previously co-director of the DNA Diagnostics Laboratory at William Beaumont Hospital in Michigan. C.J. Yu, PhD, is director of chemistry and previously conducted advanced research on low-cost DNA modification and labeling at **BioGenex Laboratories** Cynthia Bamdad, PhD, is the project manager for nucleic acid sensors and Stephen O'Connor, PhD, is project manager for engineering. —WD

clinical **micro**sensors

Exhibit 4

Clinical Micro Sensors Wins NIST ATP Award in "Tools for DNA Diagnostics" Competition

Breakthrough DNA Microchip Technology Recognized for Point of Care Applications

PASADENA, Calif.--(BW HealthWire)--Oct. 7, 1998-- Clinical Micro Sensors Inc. (CMS) Wednesday announced that the National Institute of Standards and Technology (NIST) has granted it an Advanced Technology Program (ATP) award entitled "DNA Diagnostics for the Point of Care Using Electronic Nucleic Acid Detection."

The annual competition is conducted by the Department of Commerce and administered by NIST. As one of the awardees, CMS will receive nearly $2 million for advanced development and testing of its portable, low-cost and easy-to-use DNA diagnostics systems.

"We are pleased that our DNA `chip' technology was chosen as a candidate to meet ATP's goal of accelerating the use of DNA diagnostics in a range of fields," said CMS President and CEO Jon F. Kayyem, Ph.D. "The award will help us continue to overcome the key economic and technological hurdles that have thus far limited commercialization of microchip-based DNA diagnostics."

CMS won the award through a rigorous peer-review process among a large field of competitors. Its approach to DNA diagnostic "chips" utilizes electronic detection of DNA at electrode surfaces modified with self-assembled arrays of molecular wires and insulators. The company's electronic detection and hybridization technologies have been developed to dramatically reduce costs by providing sensitive analysis using low-cost disposable chips and hand-held electronics.

The $2 million cost-sharing award will be used to conduct advanced research and development on the electronic DNA chip and hand-held instrument. Specific applications for diagnosis and detection of infectious diseases, genetic variation and pathogens in food will also be developed. Clinical testing and validation will be performed in collaboration with the UCLA School of Medicine.

ATP's Tools for DNA Diagnostics Program is intended to promote U.S. economic growth and accelerate the use of DNA diagnostics in many fields. The business goal is to promote the development of a new and very large potential market opportunity based on DNA-diagnostic systems.

Clinical Micro Sensors is a privately held company founded in 1995 to develop portable, low-cost and easy-to-use products for the electronic detection and identification of DNA and other biomolecules. Its breakthrough DNA detection technology promises to expand the range of commercial uses for molecular diagnostics. Applications include clinical diagnostics, biomedical research, food safety, environmental monitoring and the detection of biological warfare agents. CMS' headquarters and R&D laboratories are based in Pasadena. The company's Web site is at www.microsensor.com.

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Exhibit
5

Instant DNA Detection

Elizabeth K. Wilson
C&EN West Coast News Bureau

CHEMICAL
& ENGINEERING NEWS

Reprinted from
Volume 76, Number 21, Page 47

Instant DNA Detection

Systems based on electrical signals move from science fiction to reality

Elizabeth K. Wilson
C&EN West Coast News Bureau

In last year's sci-fi movie "Gattaca," the protagonist, attempting to conceal the fact that he's assumed the identity of a genetically "superior" man, scrubs himself daily to rid his body of loose hairs, skin flakes, and anything that might reveal his true identity through his DNA.

His paranoia is understandable: In the "Gattaca" future, vigilant police and employers carry handheld sensors that instantaneously analyze the DNA in snips of hair, drops of blood, or urine in order to expose the genetically "inferior." Parents receive their newborn babies' entire genetic profiles in a few minutes.

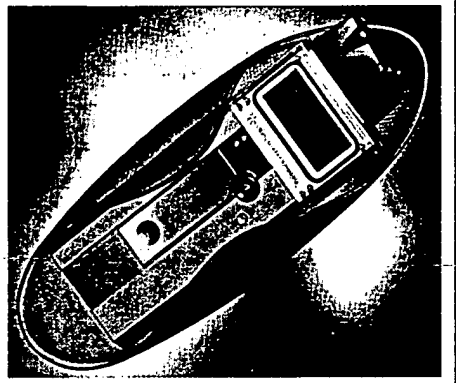
Actually, modern-day science is not far behind this technological scenario, though it envisions more humanitarian applications: detecting diseases, monitoring air for biological warfare agents, or checking food-processing plants for bacterial contamination.

Currently, extremely accurate tests for DNA sequences are based on fluorescence signals. The polymerase chain reaction (PCR) multiplies minuscule amounts of DNA into readable quantities. Although these techniques are extremely sensitive and quantitative, they require time, sample preparation, and expensive equipment. And the systems generally aren't portable.

But a number of research groups around the world are now closing in on the technology needed to develop a device that a doctor can use during an office visit or in the field to obtain results within minutes. The key to this technology is electricity.

When a single DNA strand encounters a complementary partner in a sample, it will hybridize. This hybridization can be detected by changes in an electrochemical signal—voltage or current, for example—usually through a redox-active or conducting molecule that behaves differently in the presence of a DNA hybrid than it does in the presence of a single strand.

"If there's a possibility of detecting hybridization in a rapid way using electrical signals instead of fluorescence, that's a tremendous advantage," says David L. Barker, vice president of research and business development at the instrumentation manufacturing company Molecular Dynamics in Sunnyvale, Calif. Electricity can be monitored without regard to the clarity or turbidity of a sample, and it doesn't require excitation lasers or reading by spectroscopic instruments.



Prototype handheld DNA sensor developed by CMS.

"A direct electric reading [to analyze DNA sequences] appears much more elegant and sensitive," notes Francis Garnier, of the molecular materials laboratory at the National Center for Scientific Research (CNRS) in Thiais, France.

If it were small, battery-operated, and cheap, such a device could be a boon for the diagnostics industry and bring a lot of money to its inventors.

"If they can develop a simple test, it's a huge potential market," says Michael J. Powell, new technologies director at Roche Molecular Biochemical in Pleasanton, Calif.

One company hoping to usher in this new wave of technology is Pasadena, Calif.-based Clinical Micro Sensors (CMS), which unveiled a prototype handheld DNA sensor at the Council on Competitiveness' National Innovation Summit, held at Massachusetts Institute of Technology in March.

CMS was founded by Thomas J. Meade, a chemist at California Institute of Technology, and Jon Faiz Kayyem, president of CMS and Meade's former postdoctoral researcher. Meade's lab pioneered research on the fact that double-stranded DNA conducts electricity more efficiently than single-stranded DNA does. CMS is now aggressively pursuing commercialization of the system, tackling the hurdles in this area such as cost, size, and marketing. It is now in pilot production of its handheld DNA sensor.

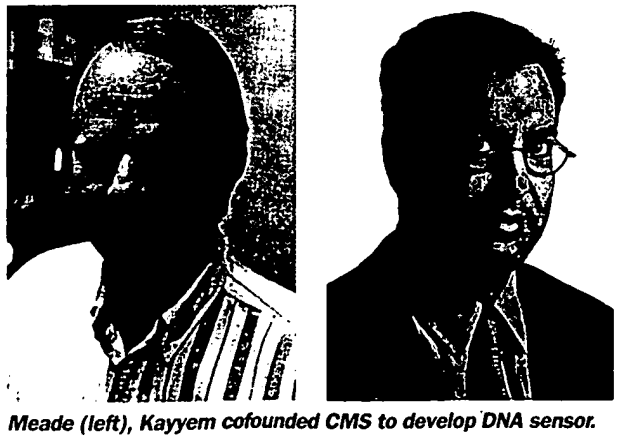
"Currently, industry can deliver accurate DNA diagnostics, fast DNA diagnostics, convenient DNA diagnostics, and, occasionally, low-cost DNA diagnostics. What it cannot do is combine these attributes into a fast, accurate, convenient, low-cost assay," Kayyem says. "That's where many of us believe biosensors will help."

New Mexico State University chemistry professor Joseph Wang, whose lab specializes in sensor research, has also developed a handheld sensor for detecting lead in blood, which he says can eventually be modified to detect DNA.

Garnier's lab, as well as those of Susan R. Mikkelsen, associate chemistry professor at the University of Waterloo in Ontario; Shigeori Takenaka, head of the molecular systems group at Kyushu University, Fukuoka, Japan; and Peter Bäuerle, chemistry professor at the University of Ulm, Germany, are also publishing ever more ingenious DNA sensor chemistry, filing patents, and working with medical diagnostics companies.

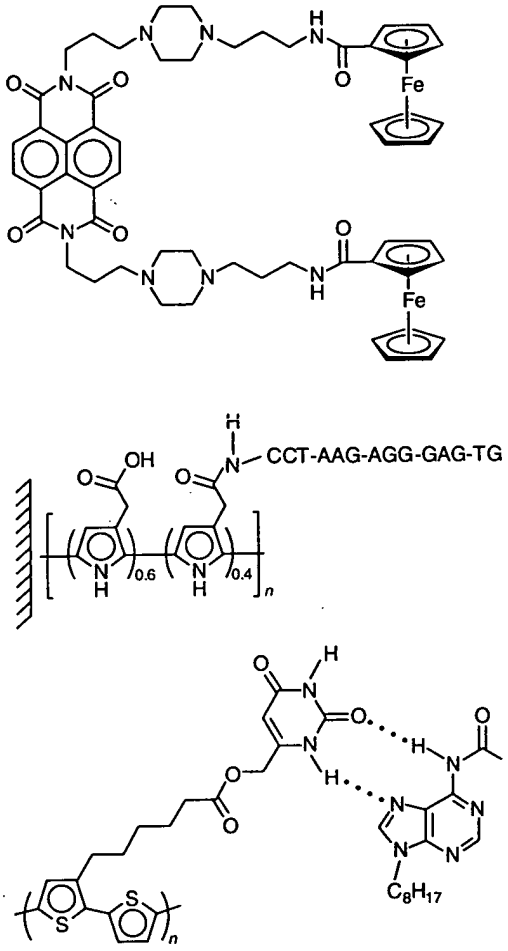
An electrochemical technique is inherently one that involves surfaces. In all the systems, DNA is bound to—"immobilized"—on a solid electrode. For example, Mikkelsen uses carbon-based material as an electrode because it's easy to generate functional groups on its surface. She then covalently binds single strands of DNA, acting as "probes," to the electrode surface. A solution containing DNA strands, known as "targets," is added. If the targets are complementary to the probes, they hybridize to form a double strand.

Then she adds a redox-active "label," in this



Meade (left), Kayyem cofounded CMS to develop DNA sensor.

Different molecules help signal DNA hybridization



Threading intercalator (top) with ferrocenyl groups at each end inserts itself through DNA. The intercalator dissociates much more slowly in the presence of double-stranded DNA [Takenaka's approach]. Polypyrroles, which are highly conductive in water, are functionalized with oligonucleotides (center). They show decreased current intensity when the oligonucleotide hybridizes [Garnier's approach]. Conducting polythiophenes functionalized with a single nucleobase (bottom) bind with the complementary nucleobase and produce a change in conductivity [Bäuerle's approach].

case a cobalt-polypyridine complex, that binds preferentially to double-stranded DNA. A higher concentration of complexes migrates to the area surrounding the hybridized DNA, which generates a current that's proportional to the local concentration of the complex.

Each lab has a different approach to DNA label or electrode design, but the basic principle is similar, and all are trying to address the biggest chemical issues: selectivity and sensitivity.

"When you do a test where the answer is yes or no, then you have to be very careful about false positives and negatives," says Mikkelsen. All sources of interference—that is, when a DNA strand matches with something other than its intended partner—need to be eliminated.

Parameters such as temperature can affect selectivity. For instance, some DNA strands could hybridize with a mismatched strand, but they're much less stable than a strand where all the base pairs are matched. It's important to find a temperature that makes it difficult for mismatched strands to stay paired.

The detection limits of many electronic methods are currently hovering around the femtomole level—and CMS can now detect at the attomole level—but researchers are trying to lower that. "Attomole detection is acceptable for many applications, but we'd like to get down to 100 molecules to better compete with PCR," Kayyem says. Labs are also looking at ways to amplify the tiny electrical signals generated by each molecule.

Researchers use many different approaches to design a DNA sensor system. In many systems, an electroactive group that facilitates electron transfer—such as cobalt or a ferrocene complex—is attached to a molecule that preferentially binds to duplex DNA. These molecules can include intercalators, which slip between base pairs of DNA, or molecules that bind to the minor groove of DNA.

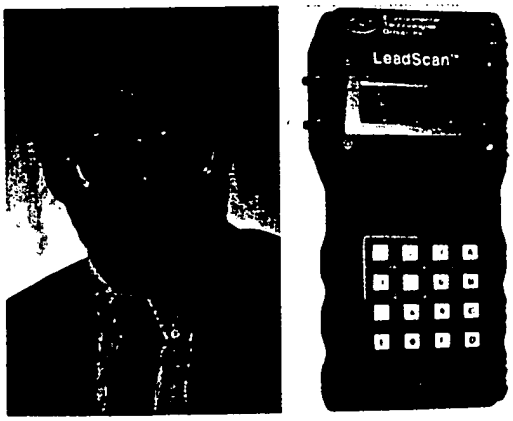
Takenaka, in collaboration with chemistry professor William David Wilson at Georgia State University, Atlanta, has developed a "threading" naphthalene diimide intercalator that actually slides through a space between DNA base pairs and out the other side. This property is useful because typical intercalators pop off, or dissociate, from a DNA strand very quickly and easily, which can make it difficult to make measurements. Takenaka's intercalator, with two bulky ferrocene groups at each end, dissociates from double-stranded DNA much more slowly and yields greater sensitivity.

Some labs are functionalizing conducting molecules with DNA. For years, Garnier's lab has studied conducting polymers with the goal of designing "intelligent materials" that sense chemicals such as enzymes and antigens or physical quantities such as photons or electric fields.

In a recent study, he grafted a 13-base oligonucleotide onto a polypyrrole, which in addition to being conductive, also shows a high degree of electroactivity in water. He found that hybridization of the oligonucleotide caused a decrease in current intensity, which he attributes to conformational changes along the polymer backbone [*J. Am. Chem. Soc.*, **119**, 7388 (1997)]. The sensitivity of their system is now 10^{-13} M. Garnier is working on improving sensitivity to 10^{-15} M. A French biomedical company is developing prototype devices based on Garnier's results, which "in terms of sensitivity, appear even more promising than expected."

Bäuerle and graduate students Andreas Emge and Alexander Meyer, who had seen changes in electrical signals when crown ethers attached to a polythiophene were complexed with alkali ions, at first studied the effect of single nucleobase-functionalized polythiophenes [*Adv. Mat.*, **3**, 324 (1998)]. They are now developing polythiophenes that are more electroactive in water and plan to do experiments with 15-base DNA strands. "We are very optimistic to see pronounced changes in the electrochemical response of the modified polymer," Bäuerle tells C&EN.

Margaret Harding and Sally Lucas, of the Australian Membrane and Biotechnology Research Institute (AMBRI) at the University of Sydney in Australia, have patented a lipid membrane biosensor in which the conductance or impedance of the membrane depends on the presence or absence of a nucleic acid sequence. Ambri Pty Ltd., which commercializes Ambri technology, is "currently considering the commercial development" of a sensor based on their work, says Stephen Conlon, business development executive at Ambri Pty Ltd. in Australia.



Wang: new concepts to enhance selectivity and sensitivity. Right: Wang's handheld sensor for detecting lead in blood.

Bäuerle (center) and graduate students Emge (left) and Meyer are developing polythiophenes that are more electroactive in water.

Wang's lab is introducing some new concepts that he hopes will enhance selectivity and sensitivity. Peptide nucleic acids, which can base-pair to DNA, can be used as probes. Because of its neutral, peptidelike backbone, peptide nucleic acid recognizes specific DNA strands even more selectively than DNA, Wang says [*J. Am. Chem. Soc.*, **118**, 7667 (1996)].

"This is crucial for obtaining mismatch discrimination, as needed for detecting point mutations," Wang says.

Wang is also developing an indicator-free sensor, based on the fact that the nucleobase guanine is easily oxidized. Hybridization can then be detected directly by an electronic signal of guanine oxidation [*Analyst*, **121**, 965 (1996)]. These detection schemes have been combined with single-use microfabricated electrodes and handheld analyzers, Wang says.

Also, binding highly branched DNA dendrimers—rather than a single strand—to an electrode can increase sensitivity, Wang says. "So far, we have over 10-fold signal enhancement and greatly improved linearity." However, he says, so far, they have used dendrimers with only 30 arms. "It's possible to design dendrimers with hundreds of arms," he adds.

The CMS system tackles the whole picture, from DNA immobilization to manufacturing. The CMS team saves preparation steps by having the label, which is usually added after hybridization, already covalently bound to the probe DNA. Samples containing whole cells and viruses can be added directly to the device, where they are split apart with heat or guanidinium isothiocyanate.

The CMS system avoids the problem of the gold electrode interacting with other redox species floating in solution by coating the electrode with a self-assembled insulating monolayer of alkane thiols. The DNA-label complexes are connected indirectly to the electrode by phenylacetylene molecular "wires," and they push through the "lawn of insulator material like dandelions," Kayyem says.

Because of the protective layer, "we can do measurements directly in very dirty environments, including blood," he adds. In addition, no washing is needed, eliminating another time-consuming step. The sensitivity of their device is on par with any current electrochemical system, Kayyem says.

The CMS system is made possible in part because of advances in DNA chip technology, where large arrays of nucleotides are attached to supports using lithography techniques. To make its electrodes, CMS buys custom-made circuit boards and immobilizes the DNA on them. Other labs are also looking into DNA chip technology. Takenaka's lab is submitting a patent on its model system with two electrodes.

In the next few years, DNA detection technology may bring the technology of "Gattaca" closer, as researchers continue to pursue the ideas that may find their way onto the DNA chips of the future.

"All ultimately may play a role in DNA diagnostics," Kayyem says.◀

M lecular 'wire' conn cts DNA c mplex t l ctrod in th CMS syst m

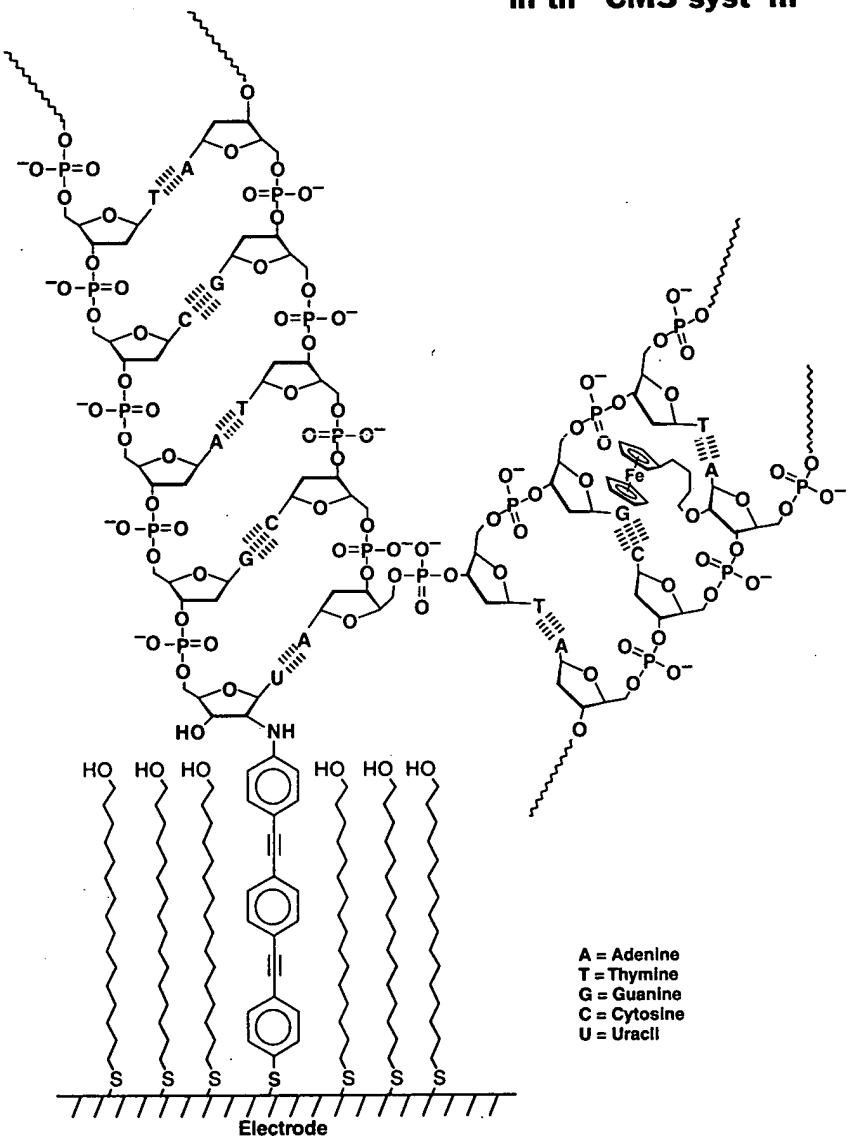


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Exhibit
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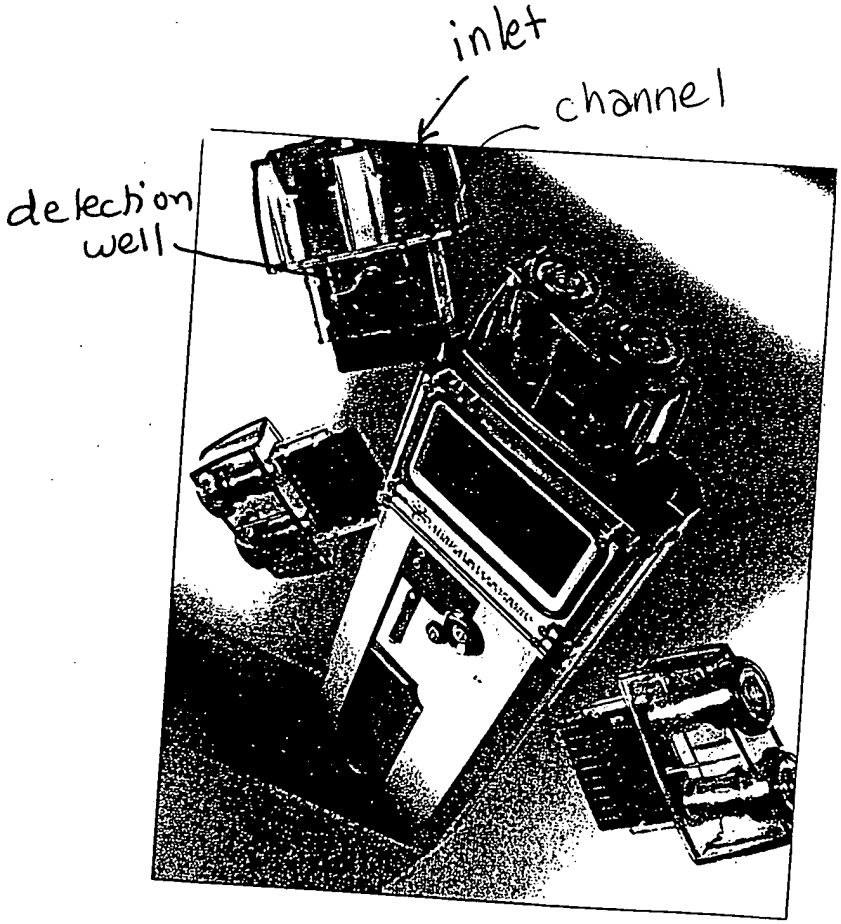

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Elmer S. Albritton
1922-1988

Exhibit
7

VIA FACSIMILE

Dr. Gary Blackburn
Dr. Faiz Kayyem
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Re: Microfluidics Patent Application
Our file: A-67465/RFT/RMS

Dear Gary and Faiz:

In regards to the draft I left for you on Friday, please review it carefully. Please note that there are a large number of questions and discussion points in bold capitals, and a number of "fill in the blanks". Rick has not yet had a chance to review this, so there will be additional changes as well.

It is particularly important that we include a complete and accurate description of your invention in the application at the time of filing since later adding new matter to clarify or further describe the invention could result in the loss of the benefit of the original filing date and require filing another application. Accordingly, if you currently have plans to modify and improve the invention or add features to the invention which are not disclosed in the draft application, these features need to be included in the patent application. Moreover, if there are any details of the manufacture of the invention which are not generally known and which are employed in the conjunction with the invention, we must disclose them.

In completing your review, please keep in mind that your invention must be described completely and in sufficient detail so that one of ordinary skill in the art can make and use the invention. Moreover, the application must describe that version of your invention which you believe is the best for achieving the intended purpose. Failure to disclose the "best mode" of practicing your invention is grounds for invalidating any patent(s) issued from the above-referenced application.

In addition, as we have discussed, I remind you of your continuing duty as an inventor to disclose to the U.S. Patent and Trademark Office (USPTO) any documents of which you are aware that are material to the patentability of your

invention. For example, such documents include issued patents (both U.S. and foreign), research articles, trade journals, advertisements, etc. which describe the prior art closest, i.e., most relevant, to your invention. If you are uncertain whether a document is "material" as used in this sense, I would be happy to assist you in making this determination. Failure to disclose these "material" documents to the USPTO can result in loss of patent rights. We will be discussing this further when we file an "information disclosure statement", but please keep it in mind.

Finally, we need to discuss inventorship on this case. My current understanding is that this is the sole invention of Faiz; let me know if there are any additional thoughts on this matter.

Please give me a call after you have had a chance to review this, and we can discuss this further.

Yours very truly,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Robin M. Silva

Enclosure
cc: Rick Trecartin (w/ encl.)

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